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Tensile Strength of Cell Walls of Living Cells[1]

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NICHOLAS C. CARPITA
Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907

ABSTRACT

A gas decompression technique was used to determine the breaking strength of cell walls of single cells. Breaking strengths of the bacterium *Salmonella typhimurium* and the unicellular green alga *Chlamydomonas eugametos* were 100 and 95 atmospheres, respectively, while those of sporophytes of the water mold *Blastocladiella emersonii* were 65 atmospheres, and those of suspension cultured cells of carrot were only 30 atmospheres. Estimation of wall tensile stress based on breaking pressures, cell radii, and estimation of wall thickness, indicates that microfibrillar walls are not necessarily stronger than walls of primitive organisms. Hence, alternative hypotheses for their evolution must be considered.

Bacterial, fungal, and plant cells are enveloped by walls, networks of polysaccharide and protein that control cell volume. Although similar in function, cell walls of different organisms differ remarkably in composition. Walls of primitive organisms vary from the chitin-like peptidoglycans of bacteria to the crystalline glycoprotein walls of the unicellular green alga, *Chlamydomonas* (8, 10, 18, 19). Walls of evolutionarily advanced fungi and plants possess multilayered microfibrillar networks of chitin or cellulose (2, 17). Although it is indisputable that walls do provide tensile strength required to maintain turgor, there are few measurements of the tensile strength of walls of single living cells because of technical problems in making such measurements mechanically. However, a nitrogen decompression technique will burst cells that are not easily disrupted by other techniques (5, 9, 13) and this N_2 gas disruption inadvertently provides a simple means to determine the average breaking pressures of the walls of single cells.

MATERIALS AND METHODS

Cell Culture. Representatives of walled cells chosen for the study were liquid cultures of the bacterium *Salmonella typhimurium*, flagellated cells of the green alga *Chlamydomonas eugametos*, developing sporophytes of the water mold *Blastocladiella emersonii*, and cell suspensions derived from callus cultures of carrot (*Daucus carota*). *S. typhimurium* stain LT2 cells were grown at 37°C to stationary phase in LB medium (4). The culture was diluted 10-fold with fresh medium, and cells were grown to o.d. 1 at 600 nm (about 2.5×10^9 cells/ml). The culture was then chilled in an ice bath to stop growth just before decompression experiments. *C. eugametos* were grown in proteose-peptose medium supplemented with acetate (20, 24). Cultures of *C. eugametos* were bubbled with sterile, humidified air, and grew to a density of 0.6 to 1.0×10^6 cells/ml. Release of zoospores of *B. emersonii* was induced by flooding cultures of mature sporophytes with water. Zoospores were transferred to aseptic stir-culture in PYG medium (15) supplemented with 1 mg/ml dextran sulfate to prevent aggregation; zoospores lost flagella, and sporophytes developed synchronously at a density of 3×10^4 cells/ml. Sporophytes enlarged to about 20 μm in diameter in about 5 h and were then chilled in an ice bath to arrest further growth just prior to decompression experiments. Cell suspensions of carrot (*D. carota L.* cV Danvers) were grown in media supplemented with 2,4-D (14), and cells at early- to mid-logarithmic phase were used in experiments (about 5 d after inoculation).

Decompression Experiments. A small beaker containing a cell suspension was placed in a stainless steel chamber (Parr Instruments), and nitrogen or nitrous oxide of up to 150 atm was introduced through a side valve. The cell suspension was stirred magnetically to hasten diffusion of the gas into the cells. After equilibration, the cell suspension was jettisoned to ambient pressure via a second valve and a tube that extended into the suspension. If the pressure differential induced by the gas plus turgor pressure was greater than the breaking strength of the wall, the cell exploded. *Salmonella* cells remaining intact after disruption were determined by plate counting of serial dilutions after incubation on LB medium containing 2% agar at 32°C overnight. For larger cells, a hemacytometer was used to quantify intact cells remaining. The *B. emersonii* sporophytes were centrifuged after disruption to concentrate the cells for counting, and small clumps of carrot cells were disaggregated in 5% chromic acid after decompression to aid counting. For all hemacytometer countings, 8 to 16 0.1 mm^2 sections containing 60 to 150 cells each were counted for each experiment, and each experiment was repeated at least three times with different cultures on different days. Values represent the mean of these experiments; values of SE for all organisms were generally about ±5%, but ranged from ±0.5% to 13%. The percentage of intact cells were plotted *versus* the pressure differential, and breaking pressures above turgor were calculated as the pressure differential required to break 50% of the population.

RESULTS AND DISCUSSION

Before analysis of data, there were technical problems to consider. Diffusivity values of nitrogen in water are rapid, about 2×10^5 $cm^2 \cdot s^{-1}$ at 22°C (22), so equilibration of an aqueous solution of 20 ml would occur within minutes. Although diffusion through the cell walls and membranes may be restricted, equilibration times in all experiments were 15 min; in pilot experiments, longer equilibration times did not decrease the relative percentages of intact cells. Conversely, release of the cell suspension to ambient pressure is very rapid, but not instantaneous, and diffusion of the gas from the cell would diminish the

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pressure differential if substantial time were required to achieve breakage. Furthermore, the surface/volume ratio of *Salmonella* cells is about 60-fold higher than that of carrot cells, so diminution of pressure by diffusion would be greater in smaller cells.

Because efflux of nitrogen also depends on the pressure differential, the slopes of the breakage curves (Fig. 1) may reflect relative diminution of the pressure gradient by diffusion. Indeed, the smaller *Salmonella* cells exhibit a shallower breakage curve (Fig. 1); however, nitrous oxide, a gas with much higher water solubility than nitrogen but similar diffusivity, produced similar breaking pressures as nitrogen for carrot cells and did not reduce survival for *Salmonella* cells up to maximum NO_2 pressures of 50 atm from that reduced by nitrogen pressure. Thus, any increase in apparent breaking pressure produced by diffusion must be small.

Another factor to consider is the extent of wall deformation before bursting because increases in surface area of the wall necessitate increases in cell volume. Young's moduli for walls of turgid plant cells are generally high enough so that substantial deformation before bursting is unlikely (17). Even so, the deformation does not depend as much on cell size as it does on diffusion. In *Salmonella* and carrot cells, stress-induced increases in surface area of 10% would require increases in volume of only 15 and 16%, respectively, even though the cells differ over 100-fold in diameter. Nitrogen pressures of up to 150 atm have little deleterious effects on the cells because gradual decompression at less than 15 atm/min resulted in cell viability of 98% for *Salmonella* cells and 92% for carrot cells, as judged by plate counting and Evans blue staining, respectively.

Breaking pressures above turgor pressure were remarkably variable among organisms. Those of *Chlamydomonas* and *Salmonella* were about 95 and 100 atm, respectively, while those of *Blastocladiella* were about 65 atm, and those of carrot were only about 30 atm (Fig. 1). Because the turgor pressure of the cells contributed to the breaking pressure, diminution of turgor by addition of NaCl or sorbitol to the incubation medium prior to disruption would be expected to increase the apparent breaking pressure of the cells. Consequently, addition of these solutes increased the percentage of cells that remained intact after a 41-atm differential (Fig. 2). The increase in protection was observed up to about the point of incipient plasmolysis (about 0.4 OsM based on plasmolytic experiments), and additional solute strength had less effect. Once turgor had been diminished, the walls had relaxed to yield a minimum cell volume, and nitrogen pressure was the same whether inside or outside the plasma membrane. In contrast, PEG 8000 continued to protect cells beyond the point of incipient plasmolysis. PEG molecules are larger than the limiting diameters of the wall capillaries, and cytorrhysis, or collapse of the wall around the shrinking protoplast, occurred rather than plasmolysis (3). Because of this collapse, nitrogen must reinflate the cell and then exert force to rupture the wall. When suspensions of carrot and *Blastocladiella* cells were incubated at incipient plasmolysis, the breaking pressure curve was shifted to a value that reflected the turgor pressure plus any volume change resulting from relaxation of wall elastic components upon loss of turgor (Fig. 3).

Figure 1 indicated that walls of more primitive organisms were actually stronger than microfibrillar walls, but this was not necessarily true. Although the pressure applied to the inner surface of the wall is independent of cell size, the stress (or tension) within the wall is a function of the radius, wall thickness, and geometric shape (12, 16, 23). For spherical cells, the equatorial wall stress (σ_E) is opposite to the pressure applied over the area of an equatorial plane through the sphere; this stress is distributed only over the area of the thin wall in this plane, or approximately thickness times circumference (12, 16, 23). This equation simplifies to:

$$\sigma_E = \frac{P \cdot r}{2t}$$

where r is the radius of the sphere, P is the hydrostatic pressure, and t is the thickness of the wall. For cylindrical cells, however, the stresses differ directionally; the tangential stress (σ_T), which limits radial expansion of the cylinder is roughly twice the longitudinal stress (σ_L; equal to σ_E) which limits elongation of the cylinder (12, 16, 23):

$$\sigma_T = \frac{P \cdot r}{t} \qquad \sigma_L = \frac{P \cdot r}{2t}$$

Wall thickness is misleading because the term belies the chemical nature of monolayer or lamellate walls, so only approximations can be made to estimate the magnitude of these wall stresses (Table I). Stresses were sizable compared to breaking pressure, and range from σ_E of only about 700 atm for nearly spherical *Blastocladiella* cells to almost 8400 atm for σ_T in *Salmonella* walls. If the equatorial stress in the carrot walls is borne by only about 50% of the width, which is plausible (17, 23), the walls would still have a tensile strength only about equal to the

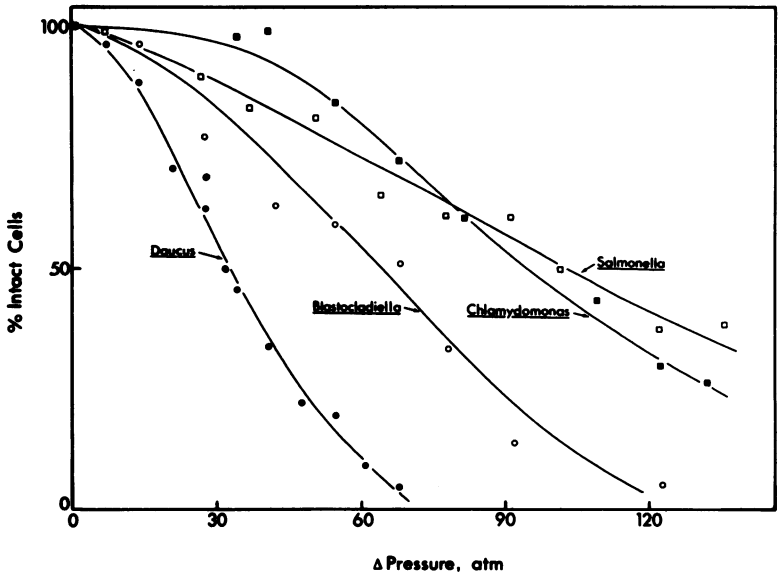

FIG. 1. Comparison of percentages of representative walled cells remaining intact *versus* the pressure differential produced by decompression of nitrogen pressurized cells.

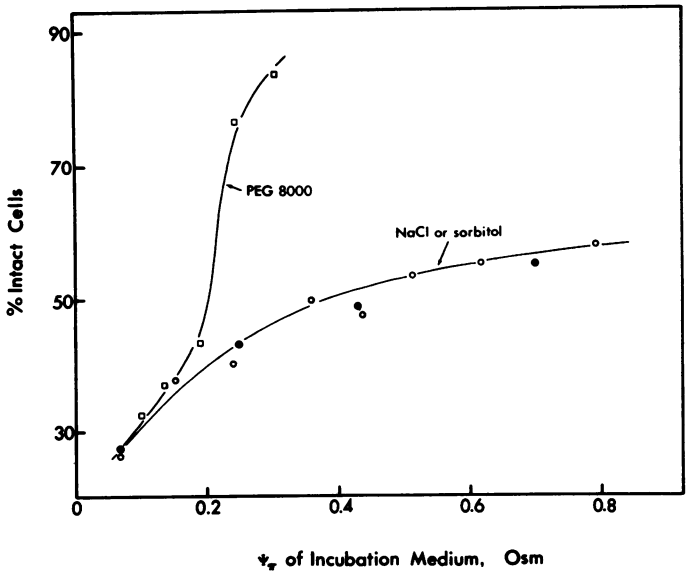

FIG. 2. Influence of cell turgor on breaking pressures of walled cells. Percentages of intact cells of *D. carota* increase when incubated in various osmotic solutions to reduce cell turgor pressure. The nitrogen-induced pressure differential was 41 atm, and PEG 8000 (□), sorbitol (●), and NaCl (○) were used as osmotic solutes.

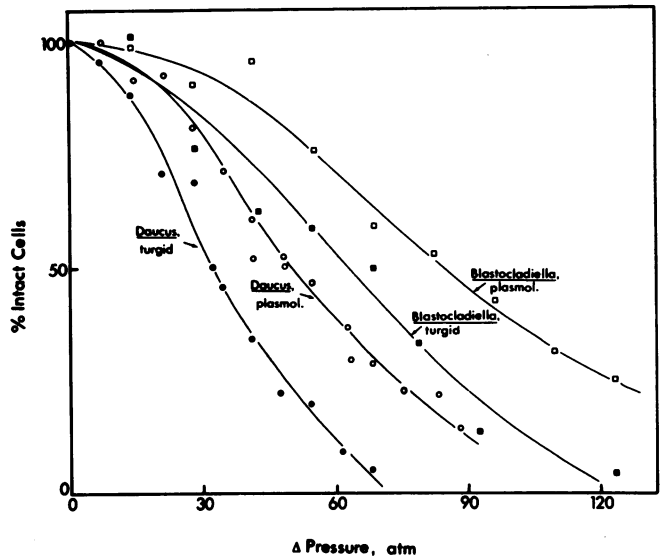

FIG. 3. Influence of pressure differential on percentages of intact cells of turgid and plasmolyzed cells of *D. carota* and *B. emersonii*. Cells of each were plasmolyzed in growth media supplemented with NaCl to 0.40 OsM. Pressure differentials were generated with nitrogen.

peptidoglycan walls of *Salmonella*.

Data acquired on these representatives of walled cells illustrate that increase in tensile strength is not the evolutionary advantage for microfibrillar walls. Obviously an increase in cell size requires a thicker or multilamellate wall structure—a 3-nm monolayer of peptidoglycan, chitin, or cellulose enveloping a carrot cell 30 μm in radius would be subjected to an intolerable one-half million atm of tension at the breaking pressure of 30 atm. Geometric shapes other than spherical produce differential tensile strength over the surface of the wall, and microfibril orientation may alter this tensile strength just as it alters the elastic moduli of fiber cells (7). Synthesis of microfibrils as fundamental wall units provides cells with both the means of organizing a multilamellate wall necessary to withstand the enormous tensions in walls of large cells as well as the versatility to orient these units to withstand the differential tensions that accompany, and possibly help direct, the diversity of cell shapes.

Table I. *Estimation of Cell Wall Stresses in Relation to Cell Dimensions and Breaking Pressure*

Estimations of radii and wall thickness were made from literature values and calculations from published electron micrographs of *S. typhimurium* (8, 21), *C. reinhardi* (18), *B emersonii* (1, 11, 15) and *D. carota* (6). Cell radii were also estimated directly from cultures of *C. eugametos*, *B. emersonii*, and *D. carota*.

Organism	Shape	Measured Breaking Pressure	Radius	Wall Thickness	Calculated Tangential or Equatorial Stress
		atm	*μm*	*μm*	*atm*
S. typhimurium	Cylindrical	100	0.25	0.003	8,400
C. eugametos	Spherical	95	8	0.060	6,300
B. emersonii	Spherical	65	10	0.45	700
D. carota	Spherical	30	30	0.1	4,500

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